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# Fine Structure of the Apical Ganglion and Its Serotonergic Cells in the Larva of *Aplysia californica*

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**Abstract.** The apical ganglion is a highly conserved structure present in various marine invertebrate larvae. Although one of the hallmarks of this ganglion is the presence of serotonergic cells, little is known about the structure and function of these cells. We have examined this ganglion in larvae of the marine mollusc *Aplysia* with light- and electron-microscopic immunocytochemistry. The results indicate that the cellular composition of the apical ganglion of *Aplysia* is very similar to that of other opisthobranchs. It consists of three classes of sensory cells (ampullary, para-ampullary, and ciliary tuft cells) and of other nerve cell types. Almost a third of the cells in the apical ganglion of *Aplysia* are serotonergic, and these can be divided into two classes: three para-ampullary and two interneuronal cells. All of the serotonergic cells extend an axon into the central nervous system. The variety of sensory and serotonergic cell types suggests that each type processes distinct attributes of the sensory environment. We argue that the apical ganglion, by virtue of its serotonergic cells, is well-suited to play important roles in the integration of sensory information to achieve proper motor adaptation to variable seawater conditions.

## Introduction

One of the most highly conserved neuronal structures across phyla is the apical ganglion (AG) (Nielsen, 1994). Also referred to as the apical sensory organ, the apical organ, or the cephalic sensory organ, the AG has so far been extensively described in the embryos, larvae, or both of cnidarians (Chia and Koss, 1979; Fukui, 1991), turbellarians (Lacalli, 1982; 1983), polychaetes (Lacalli, 1981; 1984), molluscs (Bonar, 1978; Chia and Koss, 1984; Page, 1992, Tardy and Dongard, 1993; Kempf and Page, 1995); brachiopods (Hay-Schmidt, 1992); phoronids (Hay-Schmidt, 1989; Lacalli, 1990), echinoderms (Bisgrove and Burke, 1986; Chia *et al.*, 1986; Nakajima, 1988; Nakajima *et al.*, 1993), and hemichordates (Dautov and Nezlin, 1992). A homologous structure may also occur in cephalochordates (Lacalli, 1994; Lacalli *et al.*, 1994). Although the precise function of this organ remains to be determined, its subcellular and cellular structures, as well as its superficial anterior position just above the mouth, have led to the suggestion that the AG is likely to be involved in sensing ambient water conditions during locomotion, feeding, and metamorphosis (Bonar, 1978; Chia and Koss, 1984).

Despite considerable variations in their fine structure, the apical ganglia of all species examined to date appear to share two characteristics: first, the presence of modified epithelial or subepithelial cells that give rise to an external tuft of nonmotile cilia; and second, the presence of serotonergic cells (Bisgrove and Burke, 1986, 1987; Nakajima, 1988; Hay-Schmidt, 1990, 1992, 1995; Kempf *et al.*, 1991; Nakajima *et al.*, 1993; Moss *et al.*, 1994; Lacalli, 1994; Kempf and Page, 1995). Despite their pervasive nature, very little is known of the structure, identity, and functions of the serotonergic neurons in the apical ganglion.

The present study describes the fine structure of the AG and its serotonergic cells in the larva of the marine mollusc *Aplysia californica*. In addition to shedding some light on the biology of the serotonergic (5HT) cells, this detailed study of the serotonergic constituents of the AG also aims at achieving a better understanding of the functions of this anatomical structure. This work is also the first to describe the presence of an apical ganglion in

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*Aplysia*. Surprisingly, despite the fact that this animal has been a favorite preparation of neurobiologists and the focus of a number of neurodevelopmental studies (Saunders and Poole, 1910; Kriegstein, 1977a, b; Schacher *et al.*, 1979a, b; Jacob, 1984), the apical ganglion has hitherto gone unnoticed in *Aplysia*.

Some of the results presented in this paper have been previously reported in abstract form (Marois *et al.*, 1992, 1993).

## Materials and Methods

### *Mariculture*

Animals were collected and maintained as described in Marois and Carew (1997a). Seven-day-old embryos (7 days after oviposition), hatchlings (9 days after oviposition), and 2-day-old larvae (11 days after oviposition) were used in this study. In addition, older (Stage 2 and 3) larvae were used for histological characterization and localization of the apical ganglion. The animals were staged according to the criteria of Kriegstein (1977a).

### *Immunocytochemistry (ICC)*

All the immunocytochemical and ultrastructural techniques were performed as described in Marois and Carew (1997a). The specificity of the serotonin antibody used has been previously demonstrated (Marois and Carew, 1997a). The results are based on semi-thin and ultra-thin sectioning of the apical ganglion of at least 10 animals, and on whole-mount processing of at least 50 animals.

*Whole-mount immunocytochemistry*. Animals were first anesthetized in a $MgCl_2$ solution isotonic to seawater for 5 min at room temperature (rt), followed by 8 min on ice. Animals were then immersed in three changes of ice-cold fixative solution (4% paraformaldehyde in Millonig's phosphate buffer saline [PBS]) for 30 min, and then left in the fixative solution at 4°C for an additional 2.5 h (total fixation time: 3 h). After five 4-min washes in PBS, Stage 1–6 larvae were exposed to trypsin (Type 1, Sigma, St. Louis, MO, 0.1% in PBS for 5 to 15 min at rt) and specimens were then immersed in 4% Triton X-100 (TX-100) in PBS for 1 h, rinsed in PBS, exposed to 10% EDTA in PBS for 45 min at rt to decalcify the shell, and rinsed in PBS. This was followed by pre-incubation in 2% goat serum (GS), 0.5% TX-100 in PBS for 1 h at 4°C, and by a primary (1°) Ab incubation (rabbit anti-serotonin, Incstar, Stillwater, MN; 1:650 in pre-incubation serum) for 2.5 days at 4°C on a shaker. The animals were then rinsed in PBS, pre-incubated in 2% GS in PBS for 1 h at 4°C, and immersed in secondary (2°) Ab solution (fluorescein isothiocyanate [FITC]-linked goat anti-rabbit IgG, Sigma, St. Louis, MO, 1:50 in PBS with 2% GS, 0.5% TX-100) for 2.5 h at 4°C, and rinsed in PBS. The specimens were mounted in a 3:1 glycerine:PBS solution, viewed under FITC optics (excitation filter, 480 nm; barrier filter, 520 nm) on a Nikon Optiphot-2 microscope, and photographed with Ilford XP2 400 or Kodak Ektachrome 400 film.

*Sectioned tissue immunocytochemistry*. Embryos and larvae were prepared as above, with the following modifications. 2° Ab (goat anti-rabbit IgG, Cappel; 1:50 in 2% GS, 0.5% TX-100 in PBS for 2 h at rt), and 3° Ab (rabbit peroxidase anti-peroxidase (PAP), Cappel; 1:50 in 2% GS, 0.5% TX-100 in PBS for 2 h at rt). Following the PBS rinses after the 3° Ab solution, the animals were processed for horseradish peroxidase (HRP) reaction (15 min in 0.05% DAB in PBS at rt; followed by 45 min in 0.005% $H_2O_2$, 0.05% DAB in PBS), rinsed in PBS, and dehydrated in an alcohol series (50, 70, 80, 95, and 3× 100% ethanol), and infiltrated in Epon (3× propylene oxide (PO); 2:1 PO:Epon; 1:2 PO:Epon, and pure Epon). A few animals were not processed for ICC and instead were stained with the Richardson's solution (Richardson *et al.*, 1960). All animals were sectioned on a Sorvall MT-2 ultramicrotome. Sections were viewed under a Nikon Optiphot-2 microscope and photographed with Kodak T-MAX 100 film.

### *Immuno-electron microscopy*

Embryos or larvae were anesthetized as described above. They were fixed for 30 min on ice and then for 3 h at 4°C on a shaker in 4% paraformaldehyde, 0.12% glutaraldehyde, 20% sucrose in Millonig's phosphate buffer (PB). After PBS rinses, the animals were decalcified in 10% EDTA in PBS (PB with 0.9% NaCl) for 45 min at rt, exposed to 1% $NaH_2B_4$ in PBS for 1 h at rt, rinsed in PBS, exposed to 0.05% trypsin for 15 min, and rinsed in PBS. This was followed by freeze-thawing: the animals were first immersed at 4°C for 2 h in cryoprotectant (25% sucrose, 10% glycerol in 0.1 *M* PB), then subsequently dipped in liquid $N_2$-cooled iso-pentane and in liquid $N_2$, and rinsed in PBS. The 1°, 2°, and tertiary (3°) Ab incubations were performed as for sectioned tissue ICC except that the pre-incubations lasted 2 h and no TX-100 was present in the pre-incubation and incubation solutions. The HRP reaction was performed as for sectioned tissue ICC, except that a metal-enhanced DAB substrate was used (Pierce, Rockford, IL; 45–60 min incubation followed by PBS rinses). The animals were subsequently osmicated in 2% $OsO_4$ in PBS for 1 h at rt on a shaker, and dehydrated and infiltrated in Epon as described for sectioned tissue ICC. Serial silver and gold sections were cut on a Sorvall MT-2 microtome and collected serially on either Formvar-coated slot copper grids or Thin-200 copper grids (EMS, Fort Washington, PA).

The sections were viewed under a Philips 300 or Zeiss EM-10 transmission electron microscope at 80 kV.

*Ultrastructure (SEM)*

Animals were anesthetized as described above and fixed in 2.5% glutaraldehyde, 20% sucrose in 0.1 *M* PB for 30 min on ice followed by 2.5 h at 4°C on a shaker. After several PBS rinses, animals were osmicated, rinsed in PBS, decalcified in 10% EDTA in PBS, rinsed in PBS, and dehydrated and infiltrated as described above. Silver and gold sections were cut and collected as described above. The grids were then stained for 12–15 min in 3% uranyl acetate and for 5 min in 0.3% lead citrate, and viewed as described above.

## Results

*General observations*

The veliger of *Aplysia californica* possesses an apical ganglion (AG), located above and between the cerebral ganglia (Figs. 1, 2). It sits atop the cerebral commissure and is composed of 15 to 20 cells, many of which are heavily ciliated (Fig. 2). Since this structure is strikingly similar to the AG in the nudibranch *Rostanga pulchra* (Chia and Koss, 1984), we have adopted the same nomenclature to describe the components of this ganglion in *Aplysia*. As in *Rostanga*, three major types of cells were observed in the AG of *Aplysia:* (1) four ampullary cells with large, heavily ciliated lumina; (2) three para-ampullary cells that extend one or two cilia from their apical surface; and (3) two ciliary tuft cells that project numerous long cilia from their apical surface. All of these cells send anterior apical projections that follow one of three tracts (left, right, and medial tracts) to reach the epithelial surface (Fig. 3). In addition to these cell types, the AG contains a few posterior cells that do not have any apical projections, and a dense neuropilar region located posterior and medial to all of these cells (Fig. 2B). This neuropil is in contact with the underlying cerebral commissure (Fig. 2A). Immunocytochemical staining for serotonin reveals that five cells of the AG are serotonergic (the three para-ampullary cells and two posterior cells; Fig. 4A). These are the only serotonergic cells in the entire CNS of the newly hatched *Aplysia* veliger (Marois and Carew, 1997a).

*Fine structure of the major cell types in the AG*

*Ampullary cells.* These are four centrally positioned cells, each containing a large lumen densely populated with cilia (Figs. 2, 3A, 4B). Posteriorly the cells border the neuropilar region, while anteriorly their cytoplasm funnels to a constricted neck to expand again as a swelling at the epithelial surface (Figs. 3A, 4B). Numerous microvili and one or two cilia protrude externally from these swellings (Figs. 3A, 4B). These apical cilia are distinct from the cilia in the lumen. The latter are entirely contained inside the lumen and do not pierce through the epithelial surface (Figs. 3A, 4B). The bases of these internal cilia are anchored into the cytoplasm of the ampullary cells (Fig. 3B). None of the ampullary cells are serotonergic.

*Para-ampullary cells.* This set of three cells surround-

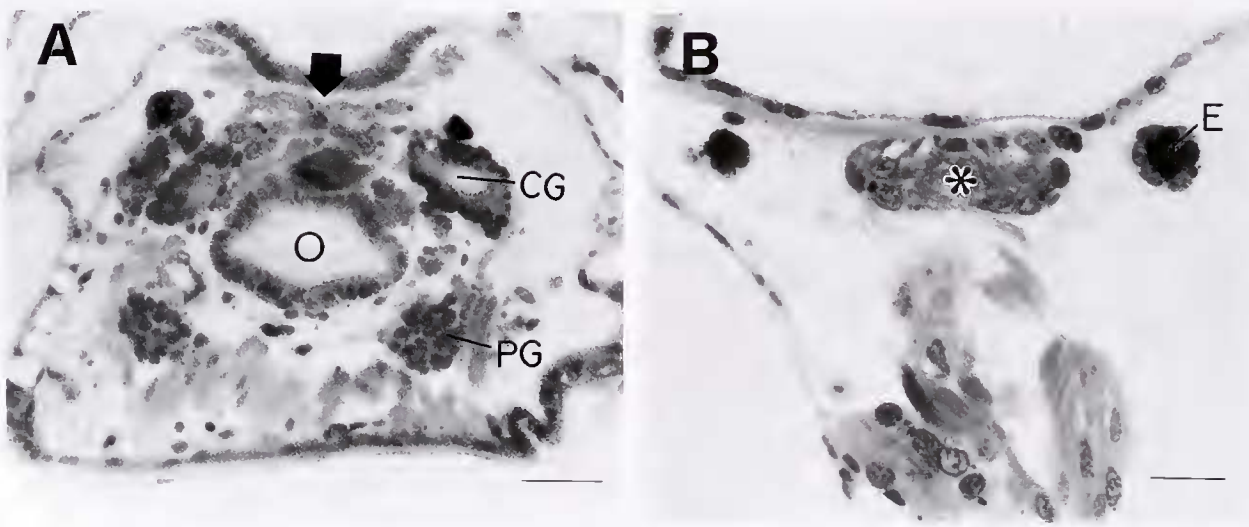


**Figure 1.** Position of the apical ganglion (AG) in *Aplysia* veligers. (A) Cross-section of a Stage 2 larva showing the AG (arrow) between the cerebral ganglia (CG) and above the oesophagus (O). (B) Horizontal section through the AG (asterisk). E, eye; PG, pedal ganglion. Scale bar: A, 20 μm; B, 15 μm.

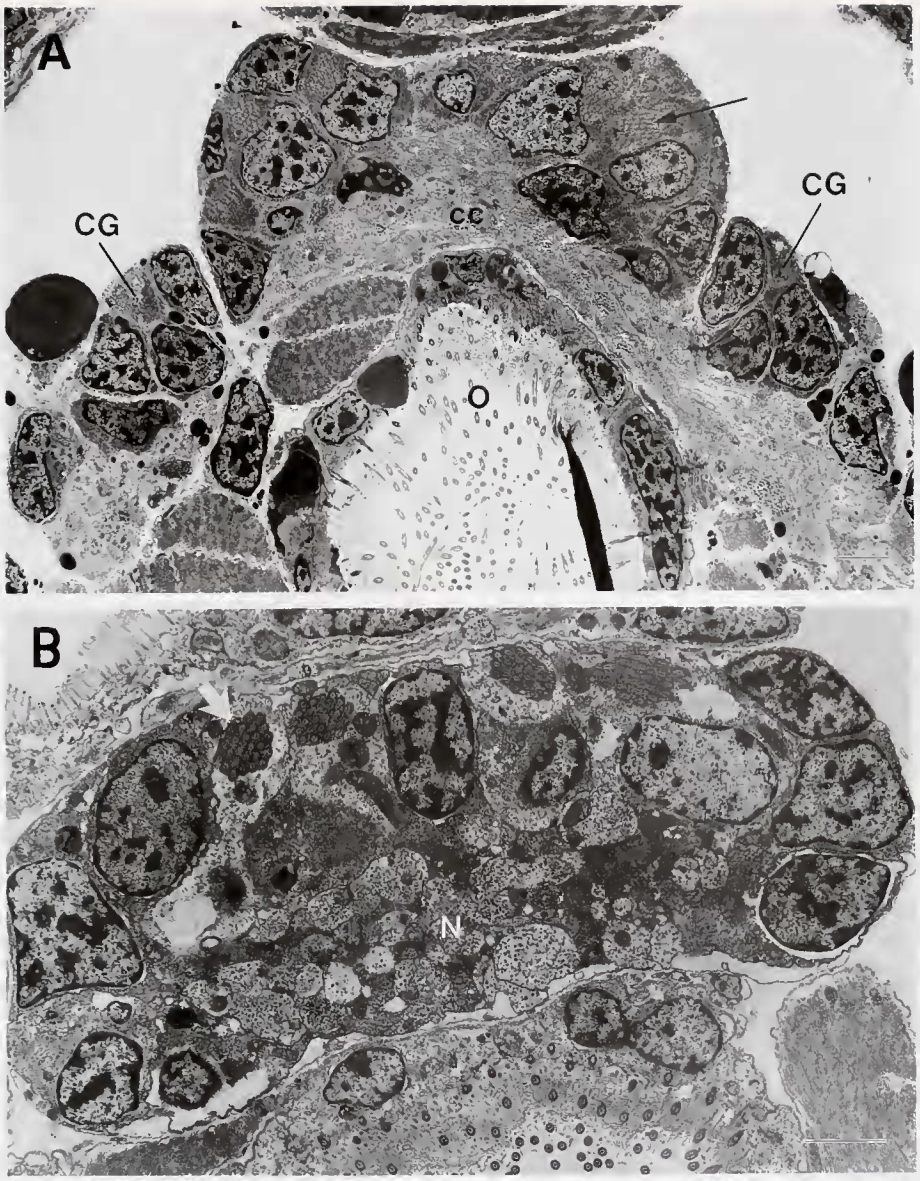


**Figure 2.** Ultrastructure of the apical ganglion. (A) Cross-section shows the AG above the cerebral commissure (cc) and between the cerebral ganglia (CG). Note the cilia (arrow) in the ampullary cells. (B) Horizontal section through the AG. Anterior is up. Note ciliary bundles (white arrow) inside the ampullary cells and a neuropil (N) posterior to these cells. O, oesophagus. Scale bar: A and B, 2 $\mu$m.

ing the ampullary cells is immunoreactive for serotonin; two of the cells are laterally positioned, and the third is centrally located between the two pairs of ampullary cells (Fig. 4A). These cells correspond to the para-ampullary cells of *Rostanga* (Chia and Koss, 1984). Each para-ampullary cell sends an anterior projection to the epithelial surface of the apical ganglion (Figs. 4, 5, 6). The projections of the lateral pair follow the lateral tracts (Figs. 4, 6), whereas the projection of the median cell emerges from the ventral side of the cell and bends anteriorly to reach the epidermal surface beneath the central tract (Fig. 5B). These three processes enlarge at the epithelial surface. From the swellings, one or two short, curly cilia extend into the external environment (Figs. 3A, 4B, 5A). The swellings also contain numerous mitochondria (Figs. 3A, 4B) and bear microvili (Fig. 3A). These expansions are linked together and to the adjacent epithelial cells by zonula adherens (Fig. 3A). In addition to their anterior projections, each of these three cells also sends a projection into the central neuropil (Fig. 4A), which appears to be heavily populated with serotonergic fibers (Fig. 5A). The distant target tissues of these central neu-

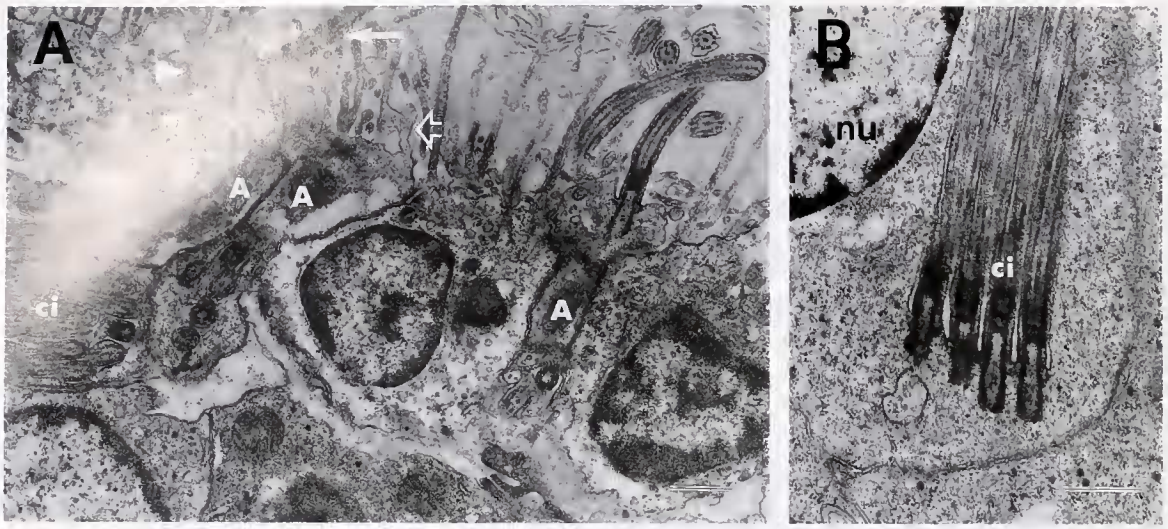


**Figure 3.** Cilia in the apical ganglion. Horizontal sections, anterior is up. (A) The apical projections of the AG cells follow tracts to the epithelial surface. The left lateral tract consists of two ampullary cell processes (A) and one para-ampullary cell process (P); the median tract has a single ampullary cell projection (A). The apical swellings of these projections extend cilia (arrow) and microvili (open arrow). The swellings are linked to each other and to the adjacent epithelial cells with zonula adherens (arrowheads). An ampullary cell contains an internal ciliary bundle (ci). (B) A ciliary bundle (ci) is attached to the cytoplasm of an ampullary cell. m, mitochondria; nu, nucleus. Scale bar: A, 0.5 μm; B, 0.5 μm.

ropilar projections have been described elsewhere (Marois and Carew, 1997b). Under conventional electron microscopy, the cytoplasm of the para-ampullary cells appears very granular and contains lipid yolk droplets, mitochondria, and small (40–60 nm) clear and dense-core vesicles (Fig. 4C). Unlike the ampullary cells, the para-ampullary cells do not contain lumina densely packed with cilia.

*Ciliary tuft cells.* A bilateral pair of rectangular cells is located at the anterior and ventral edge of the AG (Fig. 6). Each cell sends a cytoplasmic projection anteriorly into the lateral tracts, underneath the projections of the ampullary and para-ampullary cells. After narrowing in the lateral tracts, the projections expand considerably at the apical surface of the AG (Fig. 7). At least five long cilia emerge from each of these two large apical swellings (Figs. 6, 7). These cilia are anchored to the swellings by long ciliary rootlets and dense basal bodies (Fig. 7). Mitochondria are found at the bases of the cilia. The swellings are linked by zonula adherens to epidermal cells, to the serotonergic swelling of the unpaired median para-ampullary cell, and to each other.

*Other serotonergic and non-serotonergic cells.* There are six to eight other cells in the AG at hatching. Two of them are immunoreactive for serotonin (Figs. 4A, 6). These 5HT cells are located immediately posterior and slightly medial to the lateral pair of para-ampullary serotonergic cells (Figs. 4A, 6). Each of these cells extends a single process into the neuropil of the AG (Fig. 4A). Since these cells have not been identified in *Rostanga* (Chia and Koss, 1984) and since they do not extend any apical projections, they are referred to as serotonergic interneurons. Little is known about the remaining cells of the AG except that they do not appear to have any apical processes and are not immunoreactive for serotonin. The structure of the apical ganglion and of its principal cellular constituents in *Aplysia* is summarized in Figure 8.

## Discussion

### *General structure of the AG of* Aplysia *and other gastropods*

The AG of *Aplysia* is strikingly similar to the apical ganglion of the opisthobranch *Rostanga pulchra* (Chia and Koss, 1984). They both have the same number and major types of cells: four ampullary cells, three para-ampullary cells, and two ciliary tuft cells. The only notable difference is that the cell bodies and apical projections of the ciliary tuft cells are ventral to the other cells in the AG of *Aplysia*, but they seem to occupy a dorsal position in *Rostanga* (Chia and Koss, 1984).

It is intriguing that the structure of the AG in both *Aplysia* and the nudibranch *Rostanga* (Chia and Koss, 1984) differs markedly from that of another nudibranch, *Phestilla sibogae* (Bonar, 1978): Ciliary tuft cells, characterized by apical projections having many long, deeply rooted cilia, appear to be absent in *Phestilla*. Neverthe-

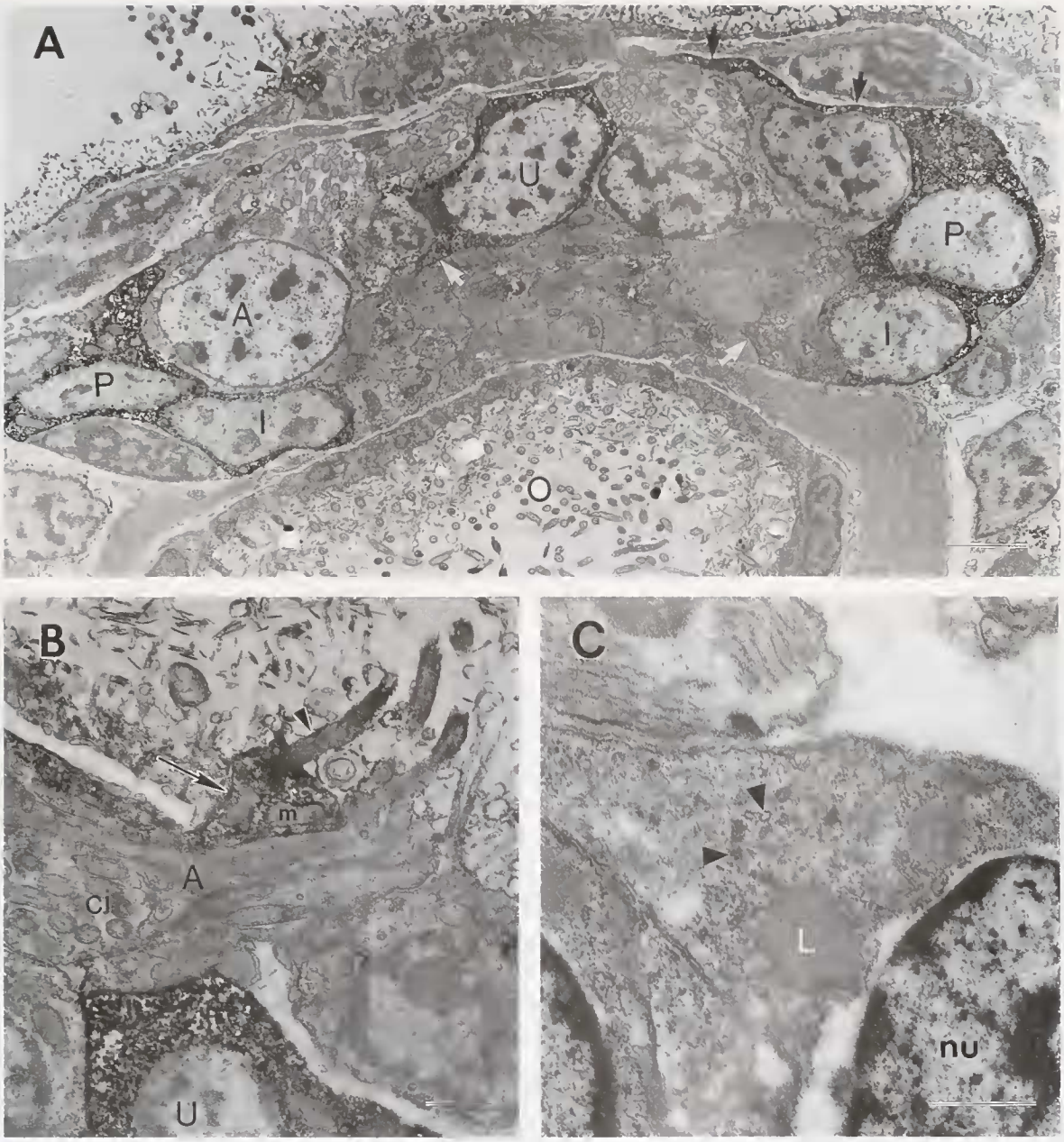


**Figure 4.** Serotonergic cells of the apical ganglion. Horizontal sections, anterior is up. (A) The lateral (P) and unpaired median (U) para-ampullary cells are serotonergic. Two interneurons (I) posterior to the lateral para-ampullary cells are also serotonergic. Note the apical projection (small black arrows) of a para-ampullary cell, the apical swelling of another (black arrowhead), and the central projections (small white arrows) of a para-ampullary cell and an interneuron. (B) Apical swelling (arrow) of a serotonergic para-ampullary cell. A cilium (arrowhead) extends from the external surface of the swelling. Note the cilia (ci) and apical process of the adjacent ampullary cell (A). (C) Fine structure of a para-ampullary cell processed for conventional electron microscopy. The cytoplasm contains lipid yolk droplets (L), and clear and dense-core vesicles (arrowheads). A, ampullary cell; ci, ciliary bundle; O, oesophagus; nu, nucleus; U, unpaired median para-ampullary cell. Scale bar: A, 2 μm; B 0.5 μm; C, 0.5 μm.

less, other cell types appear structurally similar to those of *Aplysia*. Thus, the ampullary cells of *Aplysia* and *Rostanga* are very similar to the flask-shaped cells of *Phestilla* (Bonar, 1978), with the notable difference that the ciliary bundles in *Phestilla* are not restricted to the lumen but extend to the surface of the animal. Likewise,

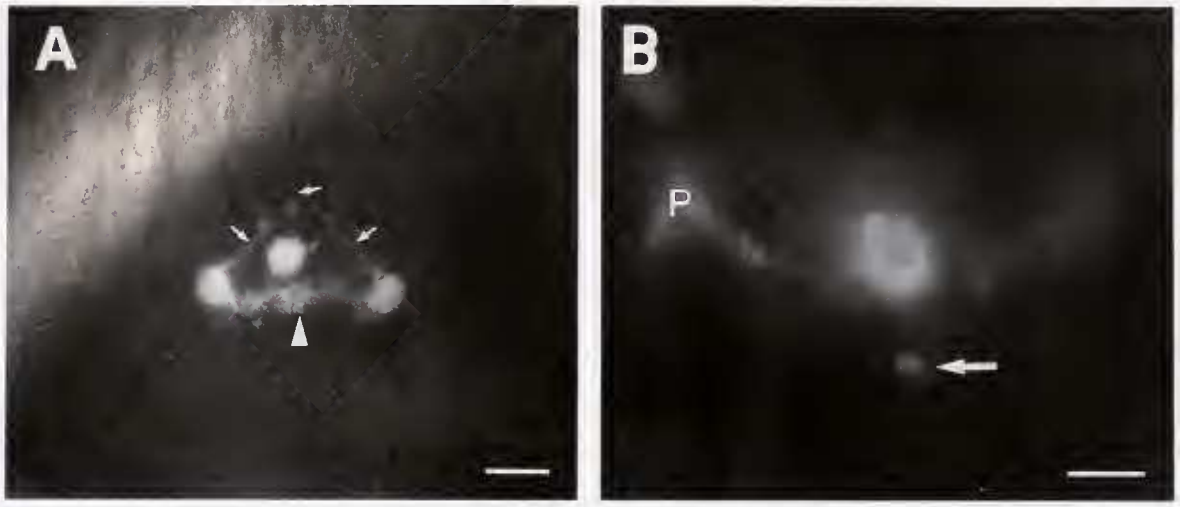


**Figure 5.** Serotonergic cells of the apical ganglion. (A) Dorsal view, anterior is up. Whole-mount immunocytochemistry in a late-stage embryo shows central projections in the neuropil (arrowhead) and apical projections with terminal cilia (small arrows) of the para-ampullary cells. (B) Frontal whole-mount ICC shows the apical projection terminating as a swelling (arrow). P, para-ampullary cell. Scale bar: A, 10 $\mu$m; B, 5 $\mu$m.

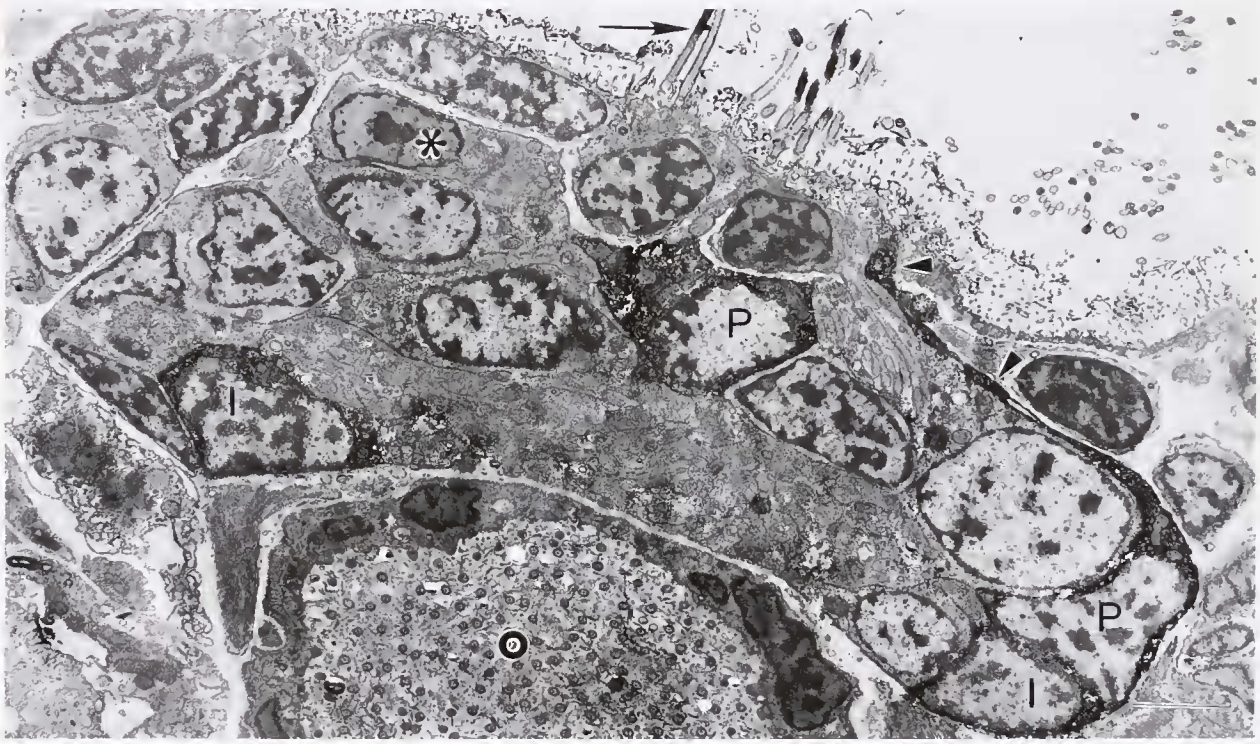


**Figure 6.** Oblique horizontal section through the apical ganglion showing two serotonergic para-ampullary (P) cells and interneurons (I) and a ciliary tuft cell (asterisk). Note the apical projection (arrowheads) of a para-ampullary cell, and the long cilia (arrow) of a ciliary tuft cell. O, oesophagus. Scale bar: 2 $\mu$m.

the cells referred to as support cells in *Phestilla* (Bonar, 1978) strongly resemble the para-ampullary cells of *Rostanga* and *Aplysia*. They surround the flask-shaped cells, and each has a narrow process extending to the surface and giving rise to microvilli and one or two cilia. Although the striking similarities between the AG cells of

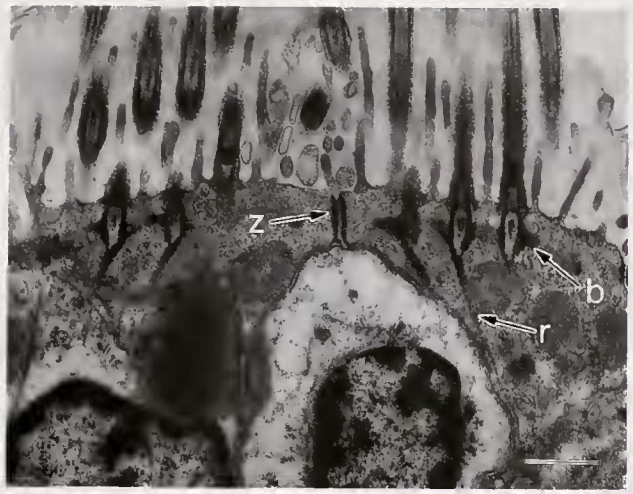


**Figure 7.** Apical projections of the ciliary tuft cells. Horizontal section, anterior is up. b, basal bodies of cilia; r, rootlet of cilia; z, zonula adherens. Scale bar, 0.5 μm.

*Rostanga*, *Phestilla*, and *Aplysia* suggest that these structures are truly homologous, more detailed phyletic studies of the AG in various prosobranchs and opisthobranchs are required before the homology is demonstrated conclusively.

### *Functions of the ciliated cells of the apical ganglion*

The morphology of the three ciliated cell types of the AG is very similar to the known morphology of epithelial chemoreceptor and mechanoreceptor cells in sensory organs of adult *Aplysia* and other invertebrates and vertebrates (Laverack, 1974; Wright, 1974; Emery and Audesirk, 1978; Altner and Prillinger, 1980; Dorsett, 1986). In addition, ultrastructural examinations of the cilia of apical ganglion cells have consistently indicated that, unlike the motile cilia of the velar cells, these cilia are nonmotile: They have a 9 + 2 microtubular arrangement lacking the dynein arms that confers motility to the cilia (Bonar, 1978; Dorsett, 1986; Nakajima, 1988). Taken together, these findings strongly suggest that the ciliated cells of the AG are sensory cells. The additional finding that these ciliated cells can be classified into three morphological groups in *Aplysia* and *Rostanga* implies that each class may serve a distinct sensory function. Chia and Koss (1984) have proposed that in *Rostanga* the internal ciliary bundles of the ampullary cells have a role in vibration or pressure detection, the long numerous cilia of the ciliary tuft cells act as distance chemoreceptors, and the short curly cilia of the para-ampullary cells act as contact chemoreceptors. Although only the para-ampullary cells were examined for central axonal projections in the present study, in *Rostanga* the three cell types extend axons into the AG neuropil (Chia and Koss, 1984). These results suggest that information about the veliger's aquatic surroundings is first gathered by the primary sensory ciliated cells of the AG and subsequently conveyed to the CNS of the animal.

### *Serotonergic cells in the apical ganglion of* Aplysia *and other gastropods*

Despite the notable differences in the structure of the AG across phyla, when serotonergic neurons have been looked for in this organ, they have invariably been found. Serotonergic neurons have been observed in the AG or apical region of the nudibranch *Berghia* (Kempf *et al.*, 1991; Kempf and Page, 1995), the prosobranch *Haliotis* (Barlow and Truman, 1992), phoronids (Hay-Schmidt, 1990), polychaetes (Hay-Schmidt, 1995), brachiopods (Hay-Schmidt, 1992) and various echinoderms (Moss *et al.*, 1994; Bisgrove and Burke, 1986; 1987; Nakajima, 1988; Nakajima *et al.*, 1993). In addition, serotonergic neurons are associated with the extreme anterior end of the amphioxus nerve cord (Holland and Holland, 1993), a region postulated to be derived from the apical organ of invertebrate ancestors (Lacalli, 1994; Lacalli *et al.*, 1994). However, it is difficult to compare the fine structure of the serotonergic cells of *Aplysia* with those of other species because these other studies have predominantly been limited to a whole-mount, light microscopic level of analysis. Nevertheless, serotonergic cells with the gross morphology of the para-ampullary cells of *Aplysia* (a short apical projection and a basal axonal process) have been observed in the apical organs of some echinoderms (Bisgrove and Burke, 1986; 1987; Nakajima, 1988), and serotonergic cells extending only basal processes, similar to the serotonergic interneurons of *Aplysia*, have been observed in polychaete and brachiopod larvae (Hay-Schmidt, 1992; 1995). Although these findings could be interpreted as indicating that the two classes of serotonergic cells observed in *Aplysia* may be differentially represented in other phyla, an examination of the fine structure of the 5HT cells in these other animal groups may reveal instead that they do not correspond to either of the two serotonergic classes in *Aplysia*.

### *Functions of the serotonergic cells in the AG*

The serotonergic cells make up about a third of the entire cellular population of the AG. This sheer number suggests that serotonergic cells are important components of the AG. Furthermore, the distinct morphology of the para-ampullary and interneurons suggests that these two types of serotonergic cells serve different functions. As mentioned above, the three para-ampullary serotonergic cells are probably sensory (Chia and Koss, 1984). Because the serotonergic interneurons do not possess an apical process or any cilia, it is unlikely that they

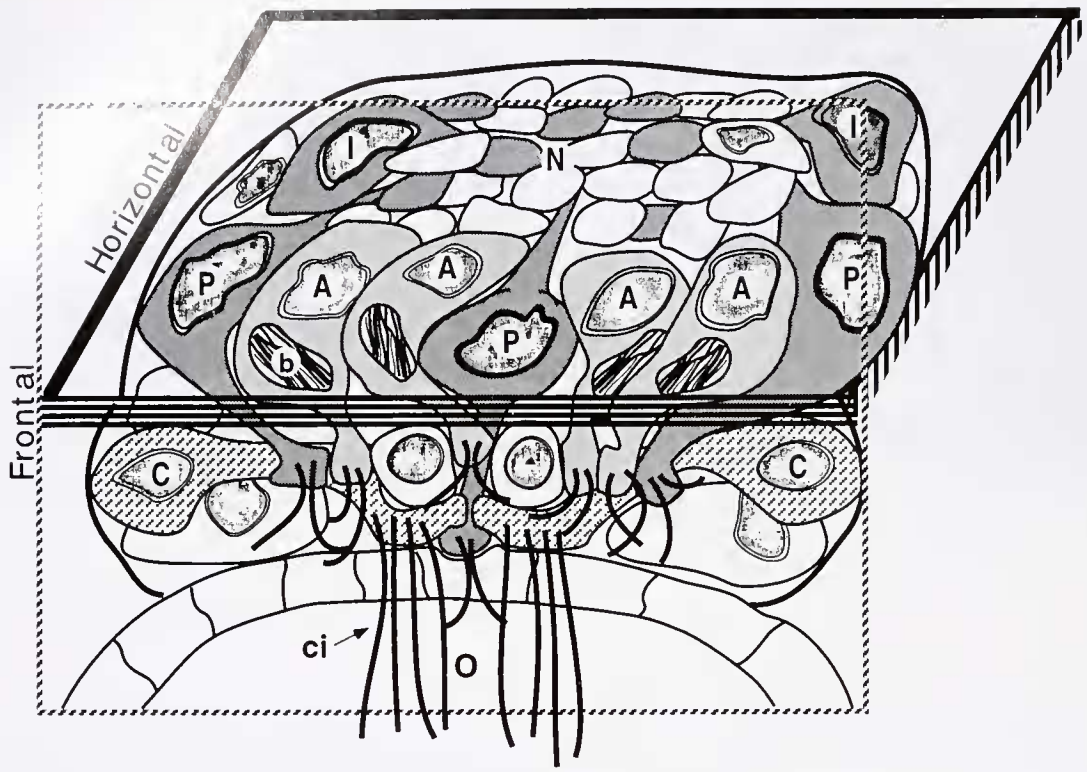


**Figure 8.** Schematic diagram of the apical ganglion of larval *Aplysia*. The diagram illustrates both horizontal and frontal plane views. The three para-ampullary (P) and the two interneuron (I) cells are serotonergic. All 5HT cells send projections into the neuropil (N). The ampullary (A) and the ciliary tuft (C) cells are also illustrated. Except for the 5HT interneurons, all the labeled cell types extend apical processes to the surface of the animal. See text for details. ci, cilia; b, ciliary bundle; O, oesophagus.

act as sensory receptors. Instead, they may act as interneurons along the information pathway that links the AG to the rest of the CNS and to effector tissues. These cells, as well as the para-ampullary cells, extend an axonal process into the AG neuropil and the cerebral commissure; these processes subsequently course in various directions to reach and innervate muscles, nerve cells, and ciliated cells of the velum (Marois and Carew, 1997b). There is circumstantial evidence that the para-ampullary cells provide the serotonergic input to the velum and the interneurons innervate the CNS of *Aplysia* (Marois and Carew, 1997b). There is also biochemical evidence from bath-application experiments that serotonin exerts a potent modulatory effect on ciliary activity and locomotion of various molluscs (Koshtoyants *et al.*, 1961; Diefenbach *et al.*, 1991; Marois and Hofstadter, unpubl. obs.). Given that these velar cells are directly contacted by serotonergic varicosities (Marois and Carew, 1997b), it is very likely that the modulatory effects of 5HT on ciliary beating are mediated by these serotonergic synapses. Thus, the serotonergic cells of the AG cannot be regarded as strictly sensory or interneuronal since they appear to have direct effector functions on ciliary activity, and probably on muscular and neuronal activity as well.

These findings suggest that the serotonergic cells in larval *Aplysia* are multimodal neurons involved in the modulation of ongoing physiological and behavioral activity (see Marois and Carew, 1997b). Although there is evidence from studies on the gastropod *Ilyanassa* that serotonin may also be involved in the induction of metamorphosis (Levantine and Bonar, 1986; Leise, 1996; Couper and Leise, 1996), no comparable effect has been observed in *Aplysia* and other gastropods (Morse *et al.*, 1979; Hadfield, 1984; Coon *et al.*, 1985; Marois and Hofstadter, unpubl. obs.).

In conclusion, this study, together with other recent work (Marois and Carew, 1997a, b), has begun to reveal the anatomical and functional properties of the serotonergic system in the apical ganglion of the gastropod *Aplysia*. However complex the functions of the serotonergic cells may turn out to be, they probably represent only a fraction of the roles played by the entire apical ganglion. The AG may therefore be best regarded as a complex nerve center for the regulation of larval-specific behaviors, integrating sensory information to achieve

proper motor adaptation to variable environmental conditions. Thus, more than simply a sensory structure, the apical organ is a *bona fide* neuronal ganglion.

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